Effect of Silibinin and VAPE Combined with 5-FU on Growth and Immune Function of Subcutaneous Transplanted Tumor in SGC7901 Bearing Mice

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Abstract: To discuss the effect of Silibinin and VA (*Cervus nippon* Temminck) protein extract (VAPE) combined with 5-FU on Growth and Immune Function of Subcutaneous Transplanted tumor in SGC7901 Bearing Mice, 24 mice were inoculated subcutaneously to establish the transplanted tumor model of gastric group and Silibinin + VA+5-FU combined group, with 6 rats in each group. After the last administration, the transplanted tumor, spleen and thymus were stripped completely. The tumor inhibition rate, NK cells,T lymphocyte, Blymhocyte in peripheral were test. iNOS was detected by Western blots. The mice in the chemotherapy group gradually showed mental depression and decreased food intake and water intake with the treatment. There was no significant difference in body mass between the groups before treatment. At the end of treatment, the body mass of nude mice in the normal saline group, the chemotherapy group, the prescription group for strengthening spleen. Compared with the chemotherapy group, the body mass of nude mice in the Silibinin + VA+5-FU group was increased. Compared with 5-FU group, CD3+, CD4+,CD8+ T cell group creased in Silibinin + VA+5-FU group. Compared with 5-FU group, iNOS expression decreased in combined group. Silibinin + VA can better inhibit the growth of subcutaneous thansplanted tumor when combined with 5-FU and improve immune status.

1. Introduction

Gastric carcinoma is a leading cause of cancerdeath worldwide [1]. In China, gastric cancer is one of the most frequently occurring cancers, with an estimated 380,000 new cases each year, accounting for more than 40% of the worldwide annual cancer incidence [2]. To date, there are almost no effective clinical methods for this highly malignant tumor. Therefore, numerous approaches have been conducted to search for efficient cancer chemopreventive and chemotherapeutic agents. It has been reported that Silibinin has antimetastatic efficacy by down-regulating TNF-alpha-induced MMP-9 expression through inhibiting the MEK/ERK pathway in gastric cancer cells [3]. However whether it could inhibit the growth of human gastric carcinoma and the underlying mechanism is still not well elucidated.

Silibinin, a flavonoid compound, has shown to be of chemopreventive potential against many cancers [4]. In our study, the results indicated that Silibinin is a cell-cycle regulator and apoptosis inducer in human gastric carcinoma SGC-7901 cells and might be used as a candidate chemopreventive agent for gastric carcinoma prevention and intervention.VAPE could effectively increase the expression levels of PI3K and Akt and activate PI3K–Akt signal pathway. These findings have provided novel insights into the further understanding of the molecular and signalling pathway mechanisms of VAPE in promoting proliferation of human peripheral blood lymphocyte cells.

In this study, MFC tumor-bearing mice were used to observe the tumor growth, peripheral blood MDSCs and immune cell levels of silymarin, VA, 5-fu and their combination, so as to understand the improvement of immune function and prognosis of patients with gastric cancer treated by the combination of traditional Chinese and western medicine, and to provide experimental basis for the development and combination of clinical treatment of tumor drugs.

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2. Materials and methods

2.1 Cell lines and culture

In this study, SGC7901 cell line was used as a HCC source and purchased from the Cell Bank and Type Culture Collection of the Chinese Academy of Sciences (Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai, China). Cells were maintained and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco/Life Technologies, Grand Island, NY, USA), 100 IU/ml penicillin, 100 IU/ml streptomycin and 2 mmol/l L-glutamine in a humidified atmosphere (5% CO2, 37oC). Cells were counted in suspensions using a Cedex analyzer (Innovatis AG, Bielefeld, Germany).

2.2 Animals

Twenty-four healthy male nude mice (weight 18–20 g, 4–5 weeks old) were obtained from the Beijing Laboratory Animal Centre (Chinese Academy of Sciences, Beijing, China. Mice were housed in a pathogen free laboratory animal center and maintained at ~22 ℃ under a 12-h dark/light cycle, and were given food and water ad libitum. All experimental procedures involving lab animals were performed according to the Institutional Animal Care and Use Committee's guidelines of Beihua University and approved by the Experimental Animal Ethics Committee of Beihua University.

2.3 MFC-bearing mouse model

Briefly, 5×10^6 SGC7901 cells were suspended in 200 μl PBS, and inoculated subcutaneously on nude mice to set up the SGC7901 tumor-bearing animal model. After 4 weeks, when tumors were approximately 4-5 mm in diameter as measured with a caliper, the mice were randomly divided into three groups of eight mice. The control group received an equal volume of PBS. Tumor sizes were monitored every two days and tumor volume was calculated using the formula: V = (length × width2)/2. After 14 days of daily injections, mice in each group were sacrificed through decapitation and the tumors were excised. Post-mortem, the body weight of the mice and the excised tumor dimensions and wet weight were determined. Subsequently, tumors were either flash frozen in liquid nitrogen or fixed in 4% paraformaldehyde overnight, followed by cryoprotection in 25% sucrose for several days. Cryoprotected tumors were then washed with 0.1% PBS prior to embedding in optimal cutting temperature (OCT) compound and preparation of 8 micron sections. For analysis of mRNA and protein expression levels in tumors, OCT compound-embedded tumor sections were scraped from glass slides of individual tumors and pooled according to treatment group to obtain sufficient tissue for further analysis. Tissues were frozen in liquid nitrogen and save at -80˚C until analysis.

2.4 Analysis of tumoral iNOS protein levels

iNOS protein expression levels in the frozen tumor tissue of the various treatment groups were quantified by western blot. Tumor tissue were cut into small pieces and weighted, and washed twice with pre-cooled PBS. Tumor tissue was treated with RIPA buffer (50 mmol/l Tris (pH 8.0), 150 mmol/l NaCl, 0.1% SDS, 1% NP40 and 0.5% sodium deoxycholate) containing protease inhibitors (1% cocktail and 1 mmol/l PMSF). Tumor tissue was homogenized each time interval of an ice bath for 1 minute to complete lysis organization with a homogenizer for 30 seconds each low-speed homogenizer. Lysate was centrifuged 14,000g with ice-cold centrifuge for 15 minutes. The supernatant was immediately transferred and save save at -80˚C until analysis. Total protein extracts were separated on 15% SDS-PAGE and transferred to PVDF membranes. The membrane was blocked with Tris-buffered saline containing 0.1% Tween 20 (pH 7.6, [TBST]) for 1 h at room temperature. Subsequently, the PVDF membrane was immunoblotted overnight at 4 ºC with the first antibody (see below) solution (1:1000). After washing twice with TBST, the membrane was incubated with HRP-labeled secondary goat anti-mouse IgG_{2a}-B antibody (sc-2073) for 1 h at room temperature and thereafter washed three times with TBST. The final detection was performed with enhanced chemiluminescence (ECL) western blotting reagents (GE Healthcare, Piscataway, NJ,

USA) and the membranes were exposed to Lumi-Film Chemiluminescent Detection Film (Roche Applied Science, Rotkreuz, Schwitzerland).

Loading differences were normalized by using a monoclonal β-actin antibody against the housekeeping control β-actin. The primary antibodies used in this study included anti-iNOS (ab178945) and anti-β-actin (SC-130301) and were all acquired from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

2.5 Statistical analysis

Measurements were performed in triplicate and results are expressed as a means ± SD. One-way analysis of variance (ANOVA) or Student’s t-tests were carried out using statistical analysis software (SPSS, Chicago, IL, USA). Tumor growth over time amongst the three groups was analyzed by two-way ANOVA using Graphpad Prism version 4.0b software (Graphpad Software, Inc., La Jolla, CA, USA). Values for $P<0.05$ were considered statistically significant.

3. Results

3.1 Silibinin + VA+5-FU inhibits the growth of Transplanted tumor in SGC7901 Bearing Mice

We evaluated the effectiveness ofSilibinin + VA inSGC7901tumors grafted subcutaneously onto nude mice. Grafted tumors were treated daily with 1 μg/μl (10 μg/10 μl PBS) DRz1, DRz1^{inv} or 10 μl PBS alone for 14 days via multipoint intratumoral injections.

None of the animals subjectively showed any signs of distress or disruption of feeding or sleep cycles. Furthermore, the results show no significant differences in body weight development and therefore discomfort or disruption of bodily homeostasis due to tumor growth may have been minimal. During excision, capillaries were observed visually in the vicinity of the tumor grafts and several sprouts penetrated the excised tumors (Figure. 1).

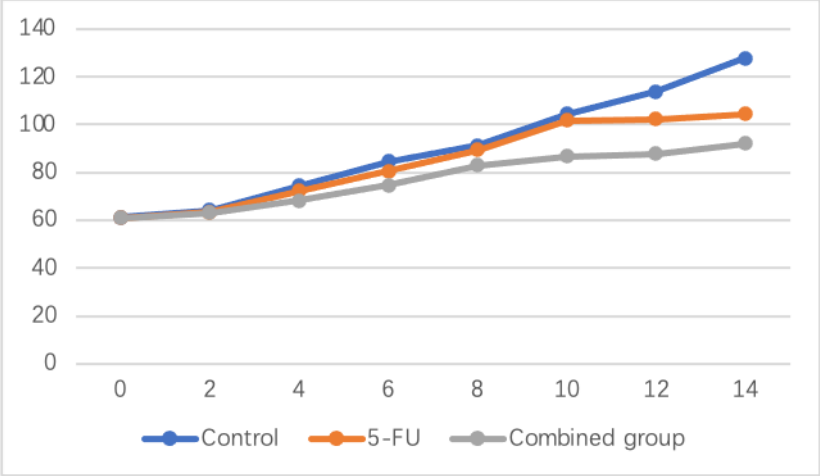

Figure 1 Effect of Silibinin + VA+5-FU on SGC7901 Bearing Mice. Tumor volume was assessed with a caliper every 2 days just befor Silibinin + VA+5-FU injection.

3.2 Silibinin + VA+5-FU reduces tumoral iNOS protein expression

To evaluate to what extent Silibinin + VA+5-FU was able to attenuate the effect of iNOS expression in the solid tumor mass, iNOS protein levels were determined. The results in Figure 2 show that Silibinin + VA+5-FU was able to significantly down-regulate the expression of iNOS in tumor, whereas no difference between the sequence inverted and sham-treated controls was observed.

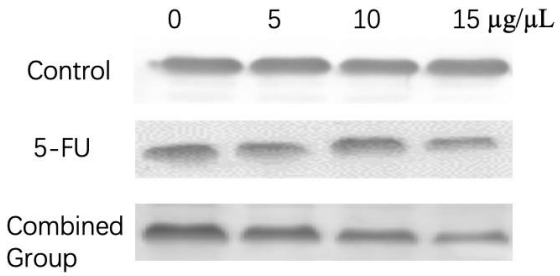

Figure 2 Effect of the Silibinin + VA+5-FU on iNOS expression in SGC7901 Bearing Mice. iNOS expression after 14 days of treatment; top: representative blot.

3.3 CD3+, CD4+,CD8+ T cell group content in Peripheral blood of mice

Compared with 5-FU group, $CD3^+$, $CD4^+$,$CD8^+$ T cell group creased in Silibinin + VA+5-FU group, $CD3^+$, $CD4^+$,$CD8^+$ T cell group decreased in 5-FU group (Figure.3).

	$CD3^+$	$CD4^+$	$CD8^+$
Control	29.67 ± 1.56	25.78 ± 1.17	17.45 ± 1.24
5-FU	25.67 ± 1.15	22.34 ± 1.89	10.87 ± 2.12
Combined group	34.89 ± 1.32	28.49 ± 1.34	19.02 ± 1.09

Figure 3 Effect of Silibinin + VA+5-FU on Tell content in Peripheral blood of mice.

4. Discussion

Cancer develops when the balance between cell proliferation and cell death is disturbed, and the aberrant cell proliferation will lead to tumor growth. It is well known that apoptosis and its related signaling pathways have a profound effect on the progression of cancer [5], suggesting that agents inducing the apoptotic death of human cancer cells may play a critical role in cancer therapy including gastric cancer. Chemotherapeutic agents induce apoptosis in most cells via two major pathways: death receptormediated pathway and mitochondria-mediated pathway. Both pathways converge to a final common pathway involving the activation of a cascade of proteases called caspases, which can cleave the regulatory and structural molecules, and thus induce the death of the cells. Surprisingly, our data demonstrated that Silibinin treatment did not show any increase in the activities of caspase 3, 8, 9 [6]. These findings clearly demonstrated the involvement of caspase-independent pathway in Silibinin-caused apoptotic death of SGC- 7901 cells. The detailed mechanism analysis is still underway in our laboratory.

Our results, generally, demonstrated that Silibinin + VA can better inhibit the growth of subcutaneous thansplanted tumor when combined with 5-FU and improve immune status, to understand the improvement of immune function and prognosis of patients with gastric cancer treated by the combination of traditional Chinese and western medicine, and to provide experimental basis for the development and combination of clinical treatment of tumor drugs.

Acknowledgements

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References

[1] Sahu Srishti,Singh Bharat & Kumar Rai Ambak.(2022).Human endogenous retrovirus regulates the initiation and progression of cancers (Review).. Molecular and clinical oncology(4),143-143.

[2] Wu Zhouqiao,Yan Su,Liu Zining,Jing Changqing,Liu Fenglin,Yu Jiang... & Ji Jiafu.(2022). Postoperative abdominal complications of gastric and colorectal cancer surgeries in China: a multicentered prospective registry-based cohort study. Science Bulletin(24),2517-2521.

[3] Wang Hongjuan,Wang Hongxia,Ge Ling,Zhao Yanying,Zhu Kongxi,Chen Zhaosheng... & Guo Jianqiang.(2021).Betulinic acid targets drug-resistant human gastric cancer cells by inducing autophagic cell death, suppresses cell migration and invasion, and modulates the ERK/MEK signaling pathway.. Acta biochimica Polonica.

[4] Chapla A, Rana PS, Sivanandhan D, et al. 2003. Silibinin upregulates the expression of cyclin-dependent kinase inhibitors and causes cell cycle arrest and apoptosis in human colon carcinoma HT-29 cells. Oncogene 22: 8271 – 8282.

[5] Dai TY, et al (2011) The antiinfective effects of velvet antler of formosan sambar deer (Cervus unicolor swinhoei) on Staphylococcus aureus-infected mice. Evidence-based Complementary and AlternAtive Medicine, 1 – 9.

[6] Agarwal R. (2000). Cell signaling and regulators of cell cycle as molecular targets for prostate cancer prevention by dietary agents. Biochem Pharmacol 60: 1051 – 1059.